


interacts with α -glucans including various hydrolases, phosphorylases, α -1,4 glucanotransferases, branching enzymes, amylases, and heat-resistant hydrolases obtained from extremophiles.--

REMARKS

The amendment is submitted to insert reference to the PCT application, to remove multiple dependency from the claims and to conform the claims to the American practice.

Respectfully submitted,
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MARKED-UP VERSION OF
CLAIMS

1. A recombinant nucleotide sequence, ^{containing} ~~characterized in that it contains~~, in the 5'→3' direction, a nucleotide sequence coding for an adenosine diphosphate glucose α-1,4-glucan α-4-glucosyltransferase or starch synthase EC 2.4.1.21, or for a protein derived from this enzyme, ~~especially~~ ^{at least} by suppression, addition or substitution of ~~one or more~~ amino acids, the said enzyme or derived protein having the property of migrating to the sites of biosynthesis of the starch granules in plant cells and of attaching to the starch granules, the said nucleotide sequence coding for the enzyme or aforementioned protein being positioned upstream of a nucleotide sequence coding for a peptide or polypeptide of interest.

2. A recombinant nucleotide sequence ^{of} ~~according to~~ Claim 1, ^{wherein} ~~characterized in that~~ the nucleotide sequence coding for a starch synthase, or for a derived protein, codes for the starch synthase bound to the starch granule or GBSS present ~~in particular~~ in plants, algae or micro-algae, ~~and more especially for the isoform GBSSI, or for a protein derived from GBSS as defined in Claim 1.~~

3. A recombinant nucleotide sequence ^{of} ~~according to~~ Claim 1 ^{wherein} ~~or 2, characterized in that~~ the nucleotide sequence coding for a starch synthase, or for a derived protein, is selected from ~~the group consisting of~~

- the nucleotide sequence SEQ ID NO : 1 of the cDNA coding for the GBSSI of *Chlamydomonas reinhardtii*,
- or a fragment of the nucleotide sequence SEQ ID NO : 1 shown, ~~such as the~~ sequences in which the nucleotide of the 5' end corresponds to that located in one of the positions 1 to 186 of SEQ ID NO : 1, and in which the nucleotide of the 3' end corresponds to that located in one of the positions 1499 to 3117 of SEQ ID NO : 1,
- 20, ~~especially~~ ^{of} ~~A recombinant nucleotide sequence of claim 3 selected from the group consisting of~~
 - the sequence SEQ ID NO : 2 coding for the GBSSI of *Chlamydomonas reinhardtii* in the form of pre protein of 708 amino acids (SEQ ID NO : 3),
 - the sequence SEQ ID NO : 4 coding for the GBSSI of *Chlamydomonas reinhardtii* in the form of mature protein of 651 amino acids (SEQ ID NO : 5),
 - the sequence SEQ ID NO : 6 coding for a fragment of 438 amino acids (SEQ ID NO : 7) of the GBSSI of *Chlamydomonas reinhardtii*,

the sequence SEQ ID NO : 8 coding for a fragment of 531 amino acids (SEQ ID NO : 9) of the GBSSI of *Chlamydomonas reinhardtii*,

- ~~or~~ a nucleotide sequence derived by degeneration of the genetic code of the aforementioned nucleotide sequences, and coding for the aforementioned GBSSI of *Chlamydomonas reinhardtii*, or for an aforementioned peptide fragment of the latter,

- ~~or~~ a nucleotide sequence derived from a nucleotide sequence or fragment mentioned above, especially by substitution, suppression or addition of one or more nucleotides, and coding for a peptide sequence derived from the aforementioned GBSSI of *Chlamydomonas reinhardtii*, or derived from an aforementioned peptide fragment of the latter, and having the property of attaching to the starch granules, the said derived nucleotide sequence preferably having a homology of at least about 50%, and preferably of at least about 70%, with the aforementioned nucleotide sequence or fragment,

- ~~or~~ ^{and} a nucleotide sequence capable of hybridizing with one of the aforementioned nucleotide sequences or fragments.

4. A recombinant nucleotide sequence ~~according to one of the Claims 1 to 3,~~ ^{wherein} ~~characterized in that~~ the nucleotide sequence coding for a peptide or polypeptide of interest is selected from ~~the group consisting of~~

- those encoding biologically active peptides, especially peptides of therapeutic interest or that can be used in the agricultural and food industry, ~~and~~ ^{is one}

^{2a. A sequence of chain 4 wherein the} ~~those encoding enzymes that are able to transform starch, such as enzymes that~~ ^{is one} interact with α -glucans including various hydrolases, phosphorylases, α -1,4 glucanotransferases, branching enzymes, amylases, and ~~especially~~ heat-resistant hydrolases obtained from extremophiles ~~such as archaeobacteria that are active at~~ temperatures above 40°C.

5. A recombinant nucleotide sequence ~~according to one of the Claims 1 to 4,~~ ^{wherein} ~~characterized in that~~ it contains a nucleotide sequence encoding a cleavage site, the said nucleotide sequence being positioned between the nucleotide sequence coding for a starch synthase, or a protein derived from the latter, and the nucleotide sequence encoding the polypeptide of interest.

6. A ~~transgenic~~ ^{group consisting of} plant cells, selected from the ~~cells~~ ^{and} of plants, algae ~~or~~ microalgae, that are able to produce starch, the said cells containing a recombinant nucleotide

sequence ~~according to one of the Claims 1 to 5~~ integrated in its genome or maintained in a stable manner in its cytoplasm.

7. ^{A member of the group consisting of} Transgenic plants, algae or micro-algae, ~~or parts, especially~~ flowers, fruits,

leaves, stems, roots, seeds, ^{and} ~~or~~ fragments of these plants, algae or micro-algae, containing a recombinant nucleotide sequence ~~according to one of the Claims 1 to 5~~ integrated in the genome or maintained in a stable manner in the cytoplasm of the cells of which they are composed.

8. A fusion polypeptide, ~~characterized in that it contains~~ ^{ly}

– in the N-terminal position, a starch synthase, or a protein derived from this enzyme, especially by suppression, addition or substitution of one or more amino acids, the said starch synthase or derived protein having the property of migrating to the sites of biosynthesis of the starch granules in plant cells and of attaching to the starch granules,

– and, in the C-terminal position, a peptide or polypeptide of interest,

the C-terminal part of the amino acid sequence of the starch synthase, or of the derived protein, thus being bound to the N-terminal part of the peptide sequence of interest, the said fusion polypeptide being encoded by a recombinant nucleotide sequence ~~according to one of the Claims 1 to 5~~.

9. A fusion polypeptide ^{of} ~~according to Claim 8, characterized in that~~ ^{wherein} the starch synthase is selected from ^{the group consisting of}

– the peptide sequence SEQ ID NO:3 corresponding to the GBSSI of *Chlamydomonas reinhardtii* in the form of pre-protein of 708 amino acids,

– or a fragment of the peptide sequence SEQ ID NO:3, ^{wherein} ~~such as~~ the sequences in which the amino acid of the amino terminal end corresponds to that located in one of the positions 1 to 58 of SEQ ID NO:3, and in which the amino acid of the carboxy terminal end corresponds to that located in one of the positions 495 to 708 of SEQ ID NO:3, ^{selected from the group consisting of}

– the sequence SEQ ID NO:5 corresponding to the GBSSI of *Chlamydomonas reinhardtii* in the form of mature protein of 651 amino acids,

– the sequence SEQ ID NO:7 corresponding to a fragment of 438 amino acids of the peptide sequence of the GBSSI of *Chlamydomonas reinhardtii*,

the sequence SEQ ID NO: 9 corresponding to a fragment of 531 amino acids of the peptide sequence of the GBSSI of *Chlamydomonas reinhardtii*,

- ~~or~~ ^{and} a peptide sequence derived from an aforementioned peptide sequence or fragment, ~~especially~~ ^{at least} by substitution, suppression or addition of one or more amino acids, and having the property of attaching to the starch granules, the said derived peptide sequence preferably having a homology of at least about 60%, ~~and advantageously at least about 80%~~, with the aforementioned peptide sequence or fragment.

10. A fusion polypeptide ~~according to Claim 8 or 9, characterized in that~~ ^{of which} it contains a cleavage site positioned between, on the one hand, the starch synthase, or a protein derived from the latter, and, on the other hand, the polypeptide of interest.

11. ^A Starch granules, ~~characterized in that they contain one or more~~ ^{containing at least} fusion polypeptides ~~defined in one of the Claims 8 to 10.~~

12. A pharmaceutical composition, ~~characterized in that it contains starch granules according to Claim 11, if necessary in combination with~~ ^{comprising a} a physiologically acceptable vehicle, ~~the said granules containing one or more fusion polypeptides as defined in one of the Claims 8 to 10, the peptide of interest in the said fusion polypeptides possessing a defined therapeutic effect.~~ ^{and at least one of them}

13. A pharmaceutical composition ~~according to Claim 12, characterized in that it is in a form that can be administered parenterally, especially intravenously, or in a form that can be administered orally,~~ ^{or} ~~the diameter of the starch granules being~~ ^{wherein} between about 0.1 μ m and several tens of μ m, and the proportion by weight of the fusion polypeptides in these granules ~~being~~ ^{is} between about 0.1% and 1%.

14. A pharmaceutical composition, ~~characterized in that it contains one or more fusion polypeptides as defined in one of the Claims 8 to 10, if necessary in combination with~~ ^{ing at least} a physiologically acceptable vehicle, the peptide of interest in the said fusion polypeptides possessing a defined therapeutic effect.

15. A food composition, ~~characterized in that it contains~~^{ing} starch granules ~~according to Claim 11, the said granules containing one or more fusion polypeptides as defined in one of the Claims 8 to 10, the peptid of interest in the said fusion polypeptides being usable in the food-processing field.~~

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16. A method of preparation of starch granules ~~according to~~^{of} Claim 11, ~~characterized in that it comprises the following stages:~~^{comprising}

- transformation of plant cells, by means of a cellular host, ~~such as~~^α ~~*Agrobacterium tumefaciens*~~, transformed by a recombinant vector, ~~especially of the~~
10 ~~plasmid, cosmid or phage type, containing a recombinant nucleotide sequence~~
~~according to one of the Claims 1 to 5,~~^{of}

- obtaining plants, algac or micro-algae transformed ~~in such a way~~^{so} that their genome contains ~~one or more nucleotide sequences according to one of the Claims 1 to 5,~~^{at least}
15 ~~by in vitro culture of the aforementioned transformed host cells,~~

- ~~if necessary,~~^{optionally} fertilization and recovery of the seeds of the plants obtained in the preceding stage, and cultivation of these seeds to obtain plants of the next generation ~~and~~

- extraction of the starch granules from the plants, algae or micro-algae, ~~or from~~^{one or more of the organs consisting of}
20 ~~parts, especially flowers, fruits, leaves, stems, roots, or fragments of these~~
~~aforementioned transformed plants, algae or micro-algae, especially by sedimentation.~~

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17. A method of preparation of fusion polypeptides ~~according to one of the~~^{of}
Claims 8 to 10, ~~characterized in that it includes the implementation of the method~~
~~according to Claim 16, the said method comprising an additional stage of recovery, and~~^{by the}
25 ~~if necessary,~~^{optionally} of purification, of the fusion polypeptides from the starch granules.

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18. A method of preparation of a peptide of interest, ~~characterized in that it~~
~~includes the implementation of~~^{by} the method ~~according to Claim 16 or Claim 17, the said~~^{of}
~~method being carried out by transformation of host cells with the nucleotide sequences~~
30 ~~according to Claim 5, and includes an additional stage of cleavage of the fusion~~
~~polypeptide obtained, by means of a suitable reagent, then, if necessary,~~^{optionally} a stage of
purification of the polypeptide of interest.

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19. A method of biotransformation of starch granules, ~~characterized in that it~~^{comprising}
~~comprises the following stages:~~

- transformation of plant cells, by means of a cellular host, ~~such as~~ ^{or} *Agrobacterium tumefaciens*, transformed by a recombinant vector, ~~especially of the~~ ^{plasmid, cosmid or phage type}, containing a recombinant nucleotide sequence according to Claim 4, encoding enzymes that are able to transform starch,
- obtaining plants, algae or micro-algae transformed ~~in such a way~~ ^{so} that their genome contains ~~one or more~~ ^{at least} aforementioned nucleotide sequences, by *in vitro* culture of the aforementioned transformed host cells,
- ~~if necessary~~ ^{or hardly}, fertilization and recovery of the seeds of the plants obtained in the preceding stage, and cultivation of these seeds to obtain plants of the next generation,
- extraction of the starch granules from ^{one member of the group consisting of} the plants, algae ~~or~~ micro-algae, ~~or from~~ parts, ~~especially~~ flowers, fruits, leaves, stems, roots, or fragments of these aforementioned transformed plants, algae or micro-algae, ~~especially by sedimentation~~,
- ~~if necessary~~ ^{or hardly}, heating of the said starch granules to a temperature at which the peptide of interest of the fusion polypeptide is capable of being active.

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